

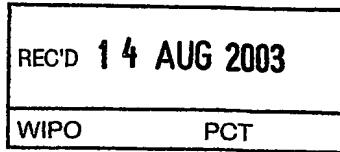


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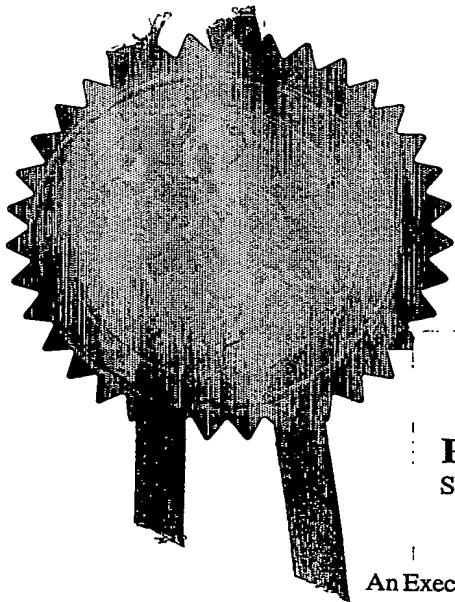
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**1. Your reference**

C237.00/Q

0216632.0

18 JUL 2002

**2. Patent application number**

(The Patent Office will fill in this part)

**3. Full name, address and postcode of the or of each applicant (underline all surnames)**

Quest International B.V.  
Huizerstraatweg 28  
1411 GP Naarden  
The Netherlands

432958 7002

Patents ADP number (if you know it)

If the applicant is a corporate body, give the country/state of its incorporation

The Netherlands

**4. Title of the invention**

Method of Reducing or Preventing Malodour

**5. Name of your agent (if you have one)**

Keith W Nash &amp; Co

"Address for service" in the United Kingdom to which all correspondence should be sent (including the postcode)

90-92 Regent Street  
Cambridge  
CB2 1DP  
United Kingdom

Patents ADP number (if you know it)

1206001

**6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and (if you know it) the or each application number**

Country	Priority application number (if you know it)	Date of filing (day / month / year)
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**7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application**

Number of earlier application	Date of filing (day / month / year)
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**8. Is a statement of inventorship and of right to grant of a patent required in support of this request? (Answer 'Yes' if:**

Yes

- a) any applicant named in part 3 is not an inventor, or
- b) there is an inventor who is not named as an applicant, or
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See note (d))

9. Enter the number of ~~sheets~~ for any of the following items you are filing with this form.  
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Description

15

Claim(s)

2

Abstract

1

Drawing(s)

-

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Priority documents

Translations of priority documents

Statement of inventorship and right to grant of a patent (Patents Form 7/77)

Request for preliminary examination and search (Patents Form 9/77)

Request for substantive examination  
(Patents Form 10/77)Any other documents  
(Please specify)

11.

I/We request the grant of a patent on the basis of this application.

Signature  
KEITH W NASH & CO  
Keith W Nash & Co., Agents

Date 18/07/2002

12. Name and daytime telephone number of person to contact in the United Kingdom

Mrs H C Matthews - (01223) 355477

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C237.00/Q

**Title: Method of Reducing or Preventing Malodour**

This invention relates to perfume components, mixtures thereof and perfume compositions, to personal products and detergent products containing such perfumes, and to the use of such perfumes and products to deliver a deodorant effect.

In particular, it relates to perfume components, mixtures thereof, and perfume compositions for inhibiting the production of odiferous metabolites by topically applying to human skin perfumery components capable of inhibiting the production of odiferous steroids by micro-organisms present on the skin surface by inhibiting 3 $\alpha$ ( $\beta$ )-sterol dehydrogenase and/or steroid 4,5-isomerase.

It is known that, at the point of secretion, sweat is odourless. Body malodour is the result of a variety of biotransformations of components of the sweat by certain species of the natural micro-organisms which live on the surface of the skin. These transformations produce volatile odiferous compounds.

There are three types of personal product routinely used to combat body malodour: perfumes, antiperspirants and deodorants.

Perfumes may simply mask body malodour. However perfume compositions have been disclosed which exhibit a deodorant action. EP-B-3172, EP-A-5618, US-A-43044679, US-A-4322308, US-A-4278658, US-A-4134838, US-A-4288341 and US-A-4289641 all describe perfume compositions which exhibit a deodorant action when applied to human skin or when included in a laundry product used to launder textiles.

Antiperspirants work by blocking the sweat glands thereby reducing perspiration.

Antimicrobial agents used in deodorants are designed to reduce the population, inhibit the growth or diminish the metabolic activities of micro-organisms living on the surface of the skin. Typical agents of this nature include ethanol and Triclosan (2',4,4'-trichloro-2-hydroxydiphenyl ether) which are well known to exert antimicrobial effects. The use of common deodorant actives results in a non-selective antimicrobial action exerted upon most of the skin's natural microflora. This is an undesirable disadvantage of such deodorant formulations, since the natural microflora provides a protective barrier against invasion by potentially pathogenic bacteria (colonisation resistance).

Gower et al. (J Steroid Biochem. Molec. Biol., (1994) Vol. 48, No. 4, pp 409-418) discloses the

importance of certain bacterial enzymes involved in bacterial steroid metabolism in the production of odoriferous steroids, and propose a series of interconversions between some of these metabolites.

US-A-5643559 (Colgate-Palmolive Company) discloses deodorant active materials having an effective amount of  $Zn^{2+}$  ions for inhibiting bacterial exoenzymes responsible for the production of axillary malodour. The bacterial exoenzymes are further characterised as aryl sulphatase or beta glucuronidase.

DE-4343265 (Henkel) describes deodorant compositions comprising saturated dioic acid (C3-C10) esters. The active inhibits a sweat decomposing esterase and the compositions are said not to disturb the skin's natural microflora.

WO 94/07837 (Unichema) describes certain novel unsaturated dioic acids having between 8 and 22 carbon atoms. The potential use of these acids to treat malodour is also described.

A number of published works describe inhibitory effects of various materials on steroid metabolic pathways:

For example, 3[[alpha]] hydroxysteroid dehydrogenase from *Pseudomonas testosteronei* is inhibited by heavy metals and sulphydryl-binding reducing agents (Talalay, P.: Hydroxysteroid Dehydrogenases in *The Enzymes*, VII, 2nd Ed., (Boyer, P., Lardy, H., and Myrback, K., eds.), Academic Press, NY, 177, 1963).

The -conazole antifungal agents have a mode of action based on inhibition of sterol metabolism. The activity of the enzyme (16-ene-C19-steroid synthesizing enzyme) responsible for the conversion of C21-steroids to 16-ene-C19-steroids, which was localized on pig testicular microsomes, was inhibited by some typical imidazole antifungal compounds such as clotrimazole, econazole, miconazole and ketoconazole which are known to be universal inhibitors of cytochrome P-450 dependent enzymes (Nakajin S, Takahashi K, Shinoda M J, J Steroid Biochem Mol Biol 1991 Jan;38(1):95-9).

20 beta-hydroxyprogrenolone is a potent inhibitor of 5,16-androstadien-3 beta-ol synthetase than of 17-hydroxylase and for the latter enzyme activity, the  $K_i$ (app) was lower than that for 17-hydroxyprogrenolone itself (Lavallee J, Cooke GM, J Steroid Biochem Mol Biol 1993 Jul;46(1):73-83).

The C16-double bond of the steroid, androsta-5,16-dien-3 beta-ol is oxidized by male rat liver microsomes to 16 alpha, 17 alpha-epoxyandrost-5-en-3 beta-ol, 16 beta, 17 beta-epoxyandrost-5-en-3 beta-ol, androst-5-ene-3 beta, 16 alpha, 17 beta-triol, and androst-5-ene-3 beta, 16 beta, 17 alpha-

triol, and this transformation is strongly inhibited with CO (Watabe T, Kornatsu T, Kobayashi K, Isobe M, Ozawa N, Saitoh Y. *J Biol Chem* 1985 Jul 25;260(16):8716-20).

However, none of the academically published literature describes the inhibition of such steroidal metabolic pathways by perfumery ingredients.

However, WO 00/01358 and WO 00/01356 disclose methods for preventing or reducing body odour by inhibiting the production of odorous steroids using agents which inhibit bacterial 4-ene reductase and/or 5-ene reductase.

Accordingly, the present invention provides a method, particularly a cosmetic method, for reducing or preventing body malodour by topically applying to human skin a composition comprising an active agent capable of inhibiting the production of odoriferous steroids by micro-organisms on the skin surface, wherein the agent is a perfume component which is capable of inhibiting  $3\alpha(\beta)$ -sterol dehydrogenase and/or steroid 4,5-isomerase.

The invention also provides the use of a perfume component to inhibit  $3\alpha(\beta)$ -sterol dehydrogenase and/or steroid 4,5-isomerase.

The invention further provides the use of a perfume composition, comprising at least 30% by weight of one or more perfume components capable of inhibiting  $3\alpha(\beta)$ -sterol dehydrogenase and/or steroid 4,5-isomerase, to reduce body malodour.

The invention further provides the use of a deodorant product, comprising a perfume component, to reduce body malodour by inhibiting  $3\alpha(\beta)$ -sterol dehydrogenase and/or steroid 4,5-isomerase.

The invention further provides a perfume composition comprising at least 30% by weight of one or more of the following perfume components: N-ethyl-N-(3-methylphenyl)propionamide, 2-ethyl-N-methyl-N-(3-methylphenyl)butanamide, dihydromyrcenol, (4-isopropylcyclohexyl)methanol, 3-methyl-5-phenylpentan-1-al; 2,2,2-trichloro-1-phenylethyl acetate, isobornyl acetate, allyl amyl glycolate,  $\alpha$ -terpineol, acetyl cedrene, tetrahydrogeraniol, citronellal, cuminic aldehyde, cis-Jasmone, pine American oil, peppermint (Chinese), 1,3,3- trimethyl-2-norbornanol, gamma-nonalactone, octahydro-2H-chromen-2-one, c/s-4-decenal, 3-(3-isopropylphenyl)butanal; and a deodorant product comprising such a perfume composition.

The invention still further provides a method of producing a perfume composition which comprises (i) evaluating perfume components on the ability to inhibit  $3\alpha(\beta)$ -sterol dehydrogenase and/or steroid 4,5-isomerase, (ii) selecting perfume components on the ability to inhibit  $3\alpha(\beta)$ -sterol dehydrogenase

and/or steroid 4,5-isomerase, and (iii) mixing together two or more of said selected perfume components, optionally with other perfume components.

The term "perfume component" is used herein to represent a material which is added to a perfume to contribute to the olfactory properties of the perfume. A perfume component can be acceptably employed to provide odour contributions to the overall hedonic performance of products. Typically, a perfume component will be generally recognised as possessing odours in its own right, will be relatively volatile and often has a molecular weight within the range 100 to 300. Typical materials which are perfume components are described in "Perfume and Flavour Chemicals", Volumes I and II (Steffan Arctander, 1969). A perfume composition will contain a number of individual perfume components, and optionally one or more suitable diluents. Commonly used diluents are benzyl benzoate, diethyl phthalate, dipropylene glycol and isopropyl myristate. The concentration of perfume components referred to herein is relative to the total concentration of perfume components present in the composition, i.e. excludes any of the above diluents.

The method according to the invention comprises topically applying to human skin active perfume components capable of inhibiting the production of odoriferous steroids by micro-organisms present on the skin surface. Preferably, the bacterial production of odoriferous steroids is reduced by at least 50%, more preferably by at least 70%, particularly by at least 80%, and especially by at least 90%. Three modes of achieving this reduction of odoriferous steroid production are possible. In the first mode, the perfume compositions may act by direct killing of skin bacteria, by more than 10-fold; in the second mode, they may act on odoriferous steroid generation whilst maintaining a microbial cell viability of at least 70%; in the third mode, they may inhibit odoriferous steroid generation, at a concentration below the minimum inhibitory concentration (MIC), determined as described in Example 1 below. The third mode is preferred, since this provides malodour counteraction benefits, whilst leaving the natural skin microflora undisturbed.

The perfume components used in the present invention are frequently incorporated into deodorant products which include, but are not limited to, body deodorants and antiperspirants including roll ons, gel products, stick deodorants, antiperspirants, shampoos, soaps, shower gels, talcum powder, hand creams, skin conditioners, sunscreens, sun tan lotions, skin and hair conditioners.

The perfume components may also be usefully employed for their deodorant properties by incorporation into other products, for example, into laundry and household products such as rinse conditioners, household cleaners and detergent cleaners. The perfume components can be incorporated into textiles themselves during their production using techniques known in the art, to provide deodorant protection.

It has been shown that the bacterial production of odoriferous steroids can be reduced or eliminated

without significantly disturbing the skin's natural microflora. This is achieved by inhibiting bacterial enzymes responsible for the production of odiferous steroids, in particular the 16-androstenes.

The odiferous steroids which are inhibited by the method of the invention include the products or intermediates of bacterial steroid metabolism, in particular the 16 androstenes, more particularly the androstadienones such as androsta-5,16-dien-3-one and androsta-4,16-dien-3-one.

In a preferred method according to the invention, an Odour Reduction Value, measured in human axillae as described in Example 4, of at least 10%, more preferably at least 30%, and particularly at least 50% is obtained. The active perfume component(s) may be mixed with other perfume components to deliver perfumes or perfume compositions with the desired deodorant and hedonistic properties. To deliver high deodorant effects the active component(s) preferably comprise(s) 30% or more of the total perfume formulation by weight, more preferably at least 40% and particularly at least 60%. A deodorant product preferably comprises at least 0.05% to 4%, more preferably 0.1% to 2% by weight of active perfume component(s). Preferred actives include the following perfume components:

N-Ethyl-N-(3-methylphenyl)propionamide (also known as 'Agarbois' where AGARBOIS is a trade mark of Quest International);

2-Ethyl-N-methyl-N-(3-methylphenyl)butanamide (also known as 'Paradisamide' where PARADISAMIDE is a trade mark of Quest International);

Dihydromyrcenol (2,6-dimethyl-7-octen-2-ol);

(4-isopropylcyclohexyl)methanol;

3-Methyl-5-phenylpentan-1-ol (also known as 'Mefrosol' where MEFROSOL is a trade mark of Quest International)

2,2,2-Trichloro-1-phenylethyl acetate (also known as Rosacetone)

Isobornyl acetate;

Allyl amyl glycolate (also known as '2-methylbutyloxyacetic acid, 2-propenyl ester');  
*alpha*-Terpineol;

Acetyl cedrene (also known as 'Lixetone' where LIXETONE is a trade mark of Quest International)  
Tetrahydrogeraniol;

Citronellal;

Cuminic aldehyde i.e. para-isopropylbenzaldehyde;

*cis*-Jasmone;

Pine American oil;

Peppermint (Chinese);

1,3,3-Trimethyl-2-norbornanol (fenchyl alcohol);  
*gamma*-Nonalactone;

Octahydro-2H-chromen-2-one (also known as 'Octahydrocoumarin' where OCTAHYDROCOUMARIN is a trade mark of Quest International);  
*cis*-4-Decenal;  
3-(3-isopropylphenyl)butanal.

A perfume composition for use in the present invention preferably comprises at least 3, more preferably at least 5, and particularly at least 10 of the above perfume components. Preferably at least 30% by weight of the perfume is comprised of perfume components possessing the ability to inhibit 3 $\alpha$ ( $\beta$ )-sterol dehydrogenase and/or steroid 4,5-isomerase, even more preferably 45%. For the purposes of calculating the percentage composition of the perfume all diluents are excluded.

One or more of the above-listed perfume components may be advantageously used in conjunction with perfumes and perfume ingredients of the prior art which offer deodorancy benefits. Examples of such embodiments comprise perfume compositions as described above which also comprise at least 15% by weight, preferably at least 30%, of the following materials: acetyl di-iso-amylene, acetyl tributyl citrate, aldehyde C10 (i.e. decenal), Amber AB 358 (available from Quest International), amyl salicylate, anisyl acetate, Azarbre\*, benzyl salicylate, *cis*-3-hexenyl salicylate, citral, citronellol, clove leaf distilled, coriander, cyclamen aldehyde, decen-1-ol, dihydroeugenol, diphenylmethane, Dupical\*, Empetaal\*, geraniol, helional [i.e. 2-methyl-3-(3,4-methylene-dioxyphenyl)propanal], ionones (alpha- and beta-), Jasmacyclene\*, 3-(4-Methyl-4-hydroxy amyl)-3-cyclohexene carboxaldehyde, methyl eugenol, methyl isoeugenol, Ortholate\*, para-cresyl methyl ether, 2-phenylethyl alcohol, para tert. butyl cyclohexyl acetate, rose oxide (racemic), styrallyl acetate, tetrahydrolinalol, and vanillin; wherein all asterisked materials are trade marks of Quest International.

Preferred embodiments include perfume compositions which contain at least 30% by weight of at least 3 of the following ingredients: N-ethyl-N-(3-methylphenyl)propionamide, 2-ethyl-N-methyl-N-(3-methylphenyl)butanamide, dihydromyrcenol, (4-isopropylcyclohexyl)methanol, 3-methyl-5-phenylpentan-1-ol; 2,2,2-trichloro-1-phenylethyl acetate, isobornyl acetate, allyl amyl glycolate, alpha-terpineol, acetyl cedrene, tetrahydrogeraniol, citronellal, cuminic aldehyde, *cis*-jasmine, pine American oil, peppermint (Chinese), 1,3,3-trimethyl-2-norbornanol, gamma-nonalactone, octahydro-2H-chromen-2-one, *cis*-4-decenal, 3-(3-isopropylphenyl)butanal; together with at least 30% by weight of one or more of the following ingredients: acetyl di-iso-amylene, acetyl tributyl citrate, aldehyde C10, Amber AB 358, amyl salicylate, anisyl acetate, Azarbre, benzyl salicylate, *cis*-3-hexenyl salicylate, citral, citronellol, clove leaf distilled, coriander, cyclamen aldehyde, decen-1-ol, dihydroeugenol, diphenylmethane, Dupical, Empetaal, geraniol, helional, alpha-ionone, beta-ionone, Jasmacyclene, 3-(4-Methyl-4-hydroxy amyl)-3-cyclohexene carboxaldehyde, methyl eugenol, methyl isoeugenol, Ortholate, para-cresyl methyl ether, 2-phenylethyl alcohol, para tert. butyl cyclohexyl acetate, rose oxide, styrallyl acetate, tetrahydrolinalol, and vanillin.

without significantly disturbing the skin's natural microflora. This is achieved by inhibiting bacterial enzymes responsible for the production of odoriferous steroids, in particular the 16-androstanes.

The odoriferous steroids which are inhibited by the method of the invention include the products or intermediates of bacterial steroid metabolism, in particular the 16 androstanes, more particularly the androstadienones such as androsta-5,16-dien-3-one and androsta-4,16-dien-3-one.

In a preferred method according to the invention, an Odour Reduction Value, measured in human axillae as described in Example 4, of at least 10%, more preferably at least 30%, and particularly at least 50% is obtained. The active perfume component(s) may be mixed with other perfume components to deliver perfumes or perfume compositions with the desired deodorant and hedonistic properties. To deliver high deodorant effects the active component(s) preferably comprise(s) 30% or more of the total perfume formulation by weight, more preferably at least 40% and particularly at least 60%. A deodorant product preferably comprises at least 0.05% to 4%, more preferably 0.1% to 2% by weight of active perfume component(s). Preferred actives include the following perfume components:

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2-Ethyl-N-methyl-N-(3-methylphenyl)butanamide (also known as 'Paradisamide' where PARADISAMIDE is a trade mark of Quest International);  
Dihydromyrcenol (2,6-dimethyl-7-octen-2-ol);  
(4-isopropylcyclohexyl)methanol;  
3-Methyl-5-phenylpentan-1-ol (also known as 'Mefrosol' where MEFROSOL is a trade mark of Quest International);  
2,2,2-Trichloro-1-phenylethyl acetate (also known as Rosacetone);  
Isobornyl acetate;  
Allyl amyl glycolate (also known as '2-methylbutyloxyacetic acid, 2-propenyl ester');  
*alpha*-Terpineol;  
Acetyl cedrene (also known as 'Lixetone' where LIXETONE is a trade mark of Quest International);  
Tetrahydrogeranial;  
Citronellal;  
Cuminic aldehyde i.e. para-isopropylbenzaldehyde;  
*cis*-Jasmone;  
Pine American oil;  
Peppermint (Chinese);  
1,3,3-Trimethyl-2-norbornanol (fenchyl alcohol);  
*gamma*-Nonalactone;

Octahydro-2H-chromen-2-one (also known as 'Octahydrocoumarin' where OCTAHYDROCOUMARIN is a trade mark of Quest International);  
*cis*-4-Decenal;  
3-(3-isopropylphenyl)butanal.

A perfume composition for use in the present invention preferably comprises at least 3, more preferably at least 5, and particularly at least 10 of the above perfume components. Preferably at least 30% by weight of the perfume is comprised of perfume components possessing the ability to inhibit  $3\alpha(\beta)$ -sterol dehydrogenase and/or steroid 4,5-isomerase, even more preferably 45%. For the purposes of calculating the percentage composition of the perfume all diluents are excluded.

One or more of the above-listed perfume components may be advantageously used in conjunction with perfumes and perfume ingredients of the prior art which offer deodorancy benefits. Examples of such embodiments comprise perfume compositions as described above which also comprise at least 15% by weight, preferably at least 30%, of the following materials: acetyl dl-iso-amylene, acetyl tributyl citrate, aldehyde C10 (i.e. decenal), Amber AB 358 (available from Quest International), amyI salicylate, anisyl acetate, Azarbre\*, benzyl salicylate, cis-3-hexenyl salicylate, citral, citronellol, clove leaf distilled, coriander, cyclamen aldehyde, decen-1-ol, dihydroeugenol, diphenylmethane, Dupical\*, Empetaal\*, geraniol, hellional [i.e. 2-methyl-3-(3,4-methylene-dioxyphenyl)propanal)], ionones (alpha- and beta-), Jasmycene\*, 3-(4-Methyl-4-hydroxy amyl)-3-cyclohexene carboxaldehyde, methyl eugenol, methyl isoeugenol, Orthololat\*, para-cresyl methyl ether, 2-phenylethyl alcohol, para terI. butyl cyclohexyl acetate, rose oxide (racemic), styrallyl acetate, tetrahydrofosalol, and vanillin; wherein all asterisked materials are trade marks of Quest International.

Preferred embodiments include perfume compositions which contain at least 30% by weight of at least 3 of the following ingredients: N-ethyl-N-(3-methylphenyl)propionamide, 2-ethyl-N-methyl-N-(3-methylphenyl)butanamide, dihydromyrcenol, (4-isopropylcyclohexyl)methanol, 3-methyl-5-phenylpentan-1-ol; 2,2,2-trichloro-1-phenylethyl acetate, isobornyl acetate, allyl amyl glycolate, *alpha*-terpineol, acetyl cedrene, tetrahydrogeraniol, citronellol, cuminic aldehyde, *cis*-jasmine, pine American oil, peppermint (Chinese), 1,3,3-trimethyl-2-norbornanol, *gamma*-nonalactone, octahydro-2H-chromen-2-one, *cis*-4-decenal, 3-(3-isopropylphenyl)butanal; together with at least 30% by weight of one or more of the following ingredients: acetyl di-iso-amylene, acetyl tributyl citrate, aldehyde C10, Amber AB 358, amyl salicylate, anisyl acetate, Azabre, benzyl salicylate, *cis*-3-hexenyl salicylate, citral; citronellol, clove leaf distilled, coriander, cyclamen aldehyde, decen-1-ol, dihydroeugenol, diphenylmethane, Dupical, Empetaal, geraniol, helional, *alpha*-ionone, *beta*-ionone, Jasmacycline, 3-(4-Methyl-4-hydroxy amyl)-3-cyclohexene carboxaldehyde, methyl eugenol, methyl isoeugenol, Ortholate, para-cresyl methyl ether, 2-phenylethyl alcohol, para tert. butyl cyclohexyl acetate, rose oxide, styrillyl acetate, tetrahydrolinalol, and vanillin.

The invention is illustrated by the following examples.

**EXAMPLE 1: MINIMUM INHIBITORY CONCENTRATION (MIC)**

The minimum inhibitory concentration of perfume components was determined by the following method.

A culture of the test strain - *Corynebacterium xerosis* NCTC 7243 (National Collection of Type Cultures, Public Health Laboratory Service, Central Public Health Laboratory, 61 Colindale Avenue, London) was grown in 100ml of tryptone soya broth (TSB) (Oxoid, Basingstoke, UK), for 16-24 hours, in a shaken flask at 37°C. The culture was then diluted in sterile 0.1% special peptone solution (Oxoid, Basingstoke, UK) to give a concentration of bacteria of approximately  $10^5$  colony forming units (cfu) per ml.

Perfume or perfume component samples were diluted in sterile TSB. Each row of a standard, 96-well plastic microtitre plate (labelled A-H) was allocated to one sample, thus eight samples per plate. Row H contained only TSB for use as a bacterial control to indicate the degree of turbidity resulting from bacterial growth in the absence of any test material. Aseptically, 200 $\mu$ l of the initial dilution of perfume/perfume component was transferred to the 1<sup>st</sup> and 7<sup>th</sup> well of the appropriate row. All other test wells were filled with 100 $\mu$ l of sterile TSB using an 8-channel micro-pipette. The contents of each of the wells in column 1 were mixed by sucking samples up and down in pipette tips, before 100 $\mu$ l was transferred to column 2. The same sterile pipette tips were used to transfer 100 $\mu$ l of each well in column 7, into the appropriate well in column 8. This set of eight tips was then discarded into disinfectant solution. Using eight fresh, sterile tips the process was repeated by transferring 100 $\mu$ l from column 2 into column 3 (and 8 into 9). The process was continued until all wells in columns 6 and 12 contained 200 $\mu$ l. After mixing, 100 $\mu$ l was discarded from wells in columns 6 and 12 to waste. Finally, 100 $\mu$ l of pre-diluted bacterial culture (approx.  $10^8$  cfu/ml) was added, thus giving 200 $\mu$ l final volume in each well.

A blank plate was prepared for each set of eight samples in exactly the same way, except that 100 $\mu$ l of sterile 0.1% special peptone was added instead of bacterial culture. Test and control plates were sealed using autoclave tape and incubated for 18 hours at 37°C.

The microtitre plate reader (Model MRX, Dynatech Laboratories) was preset to gently agitate the plates, to mix the contents. The absorbance at 540nm was used as a measure of turbidity resulting from bacterial growth. The control, un-inoculated plate for each set of samples was read first, and the plate reader then programmed to use the control readings to blank all other plate readings for the

inoculated plates for the same set of test materials (i.e. removing turbidity due to perfume and possible colour changes during incubation). Thus the corrected readings generated were absorbances resulting from turbidity from bacterial growth. The MIC was taken as the concentration of perfume/perfume component required to inhibit growth so that the change in absorbance during the incubation period was < 0.2.

#### EXAMPLE 2: STEROID BIOTRANSFORMATION ASSAY

The ability of perfumery ingredients and mixtures of these ingredients to inhibit the bacterial sterol dehydrogenase and/or steroid isomerase enzymes was determined *in vitro* using the method described below.

*Corynebacterium* sp. NCIMB 40930 (National Collections Of Industrial, Food and Marine Bacteria, 23 St Machar Drive, Aberdeen, AB24 3RY, Scotland, UK) (also known as *Corynebacterium* G41) was grown in 100ml of TSB supplemented with 0.1% w/v yeast extract (Oxoid), and 0.1% v/v Tween 80 (Sigma, Poole, UK) for 16-24 hours, in a shaken flask at 37°C. This culture was then harvested by centrifugation, and resuspended in 100 ml of biotransformation medium (consisting of a sterile semi-synthetic basal medium containing KH<sub>2</sub>PO<sub>4</sub> 1.6g/l; (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub> 5 g/l; Na<sub>2</sub>SO<sub>4</sub> 0.38 g/l; yeast nitrogen base 3.35 g/l; yeast extract 0.5 g/l; Tween 80 0.2 g/l; Triton X-100 0.2 g/l and MgCl<sub>2</sub>.6H<sub>2</sub>O 0.5 g/l).

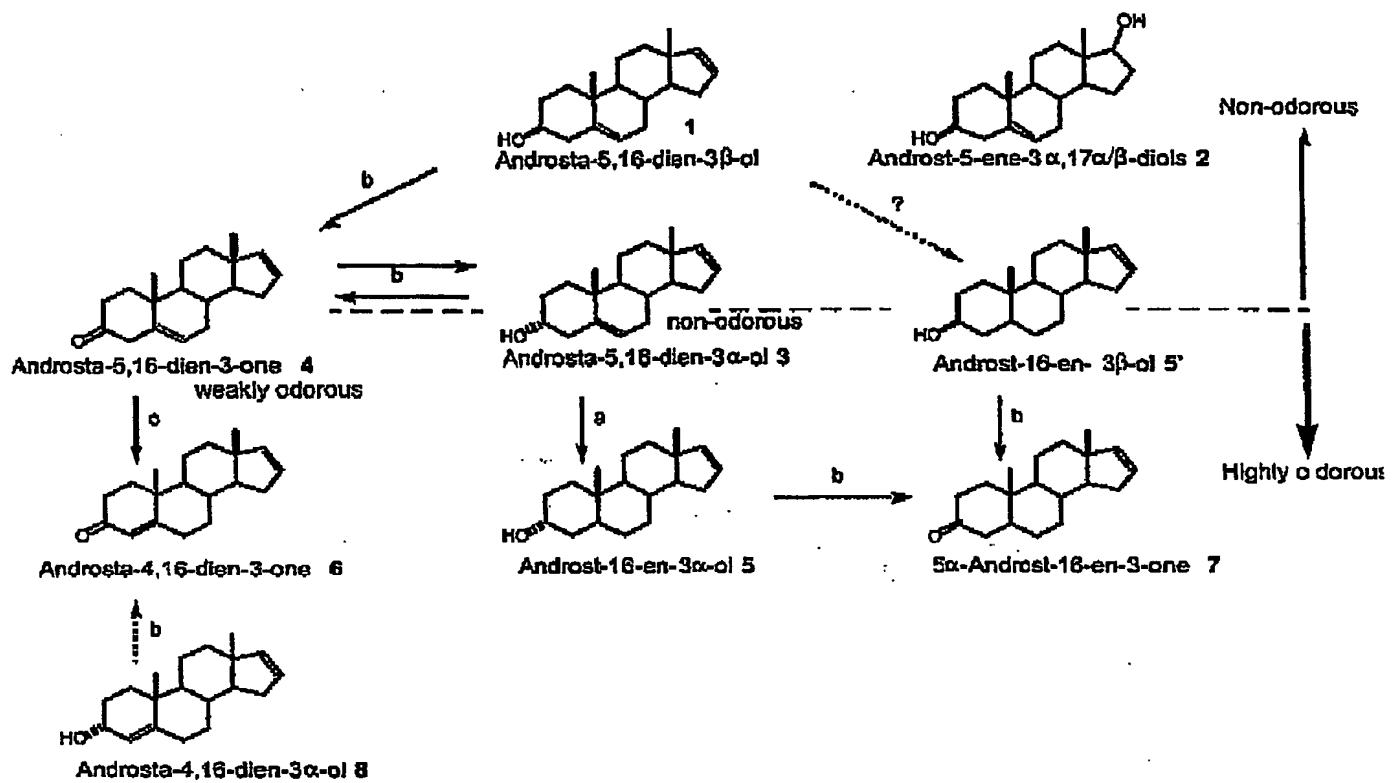
Substrate androsta-5,16-dien-3 $\beta$ -ol (50mg/assay) was added to the bacterial suspension and incubated for 72 hours at 37°C with agitation (at 220-250rpm) in a 250 ml, baffled-Erlenmeyer flask.

Following biotransformation of androsta-5,16-dien-3 $\beta$ -ol to androsta-4,16-dien-3-one the bacteria were harvested and the cell pellet dried in air and then under vacuum.

The dried cells were then crushed and suspended in a mixture of diethyl ether, chloroform, ethanol, ethyl acetate and acetone, and stirred for 16 hours. The supernatant was then reduced to half volume, filtered and evaporated at 30°C, and 15 mm Hg pressure. The resulting residue was re-dissolved in 5 ml AR grade methanol. Following sonication, the sample was analysed by HPLC on a Phenomenex Luna 5 micron, C18 reverse-phase HPLC column coupled to a Millipore-Waters 600E System Controller. Elute was passed through a Millipore-Waters 486 Tunable absorbance detector and relative amounts of the steroid metabolite was determined by a Hewlett Packard HP 3396A Integrator printer. The composition of the HPLC mobile phase was aqueous methanol. The flow rate was 0.8 ml/min. Calibration curves were used to determine the molar quantities of pure steroid metabolites in biotransformed mixtures and hence the conversions.

Metabolites were analysed by HPLC-MS to determine their structure.

Without wishing to be unduly bound or limited by theory, based upon our results, we propose the following scheme of biotransformations of steroids by *Corynebacterium* NCIMB 40930.



EXAMPLE 3: PREFERRED EMBODIMENTS

Perfume A: Composition % by weight.

INGREDIENT	w/w%
AGARBOIS (Q) **	15
CINNAMIC ALCOHOL	2
COUMARIN	1
DIHYDROMYRCENOL **	8
GERANIUM OIL	2
HABANOLIDE (F)	3
LILIAL (G)	10
(4-ISOPROPYL CYCLOHEXYL)METHANOL **	2
MEFROSOL (Q) **	5
METHYL ANTHRANILATE	1
METHYL CEDRYL KETONE	4
METHYL DIHYDROJASMONATE (Q)	10
PHENYL ETHYL ALCOHOL	15
ROSACETONE **	5
VANILLIN 5% IN DEP	17
total	100.00%

\*\* Materials of the Invention

Trademarks: 'Q' = Quest International; 'F' = Firmenich; 'G' = Givaudan

Perfume B: Composition % by weight.

INGREDIENT	w/w%
ACETYL CEDRENE	7.5 *
AGARBOIS (Q)	6 *
ALDEHYDE MNA 10% DEP	1
ALLYL AMYL GLYCOLATE (Q)	2.2 *
AMBER CORE (Q)	0.5
ARMOISE TUNISIAN	0.4
BANGALOL (Q)	0.5
BENZYL SALICYLATE (Q)	8.5
BERGAMOT OIL	7.5
BOURGEONAL (Q)	0.5
CARYONE LAEVO (Q) 10% DEP	1
CEDARWOOD VIRGINIAN OIL	1.1
cis-3-HEXENYL SALICYLATE	1.5
CISTULATE (Q) 10% DEP	2
CORIANDER	0.3
COUMARIN	0.6
CYCLOHEXYLOXYACETIC ACID, ALLYL ESTER	0.2
CYCLOPENTADECANOLIDE	2.2
DIHYDROMYRCENOL (Q)	13 *
ETHYLENE BRASSYLATE	1.5

GERANIUM OIL	1.4
HELIONAL	0.3
HEXYL CINNAMIC ALDEHYDE	2.5
IONONE (Q)	1.5
ISO AMBOIS (Q)	7.5
ISO BORNYL ACETATE	0.6
ISOBORNYL CYCLOHEXANOL	1.5
LAVANDIN OIL	0.3
LILIAL (G)	6.8
METHYL CHAVICOL	1.2
METHYL DIHYDROJASMONATE SUPER (Q)	6.4
MOSS OAKMOSS SYNTHETIC	0.2
NUTMEG PURE	0.2
PEPPERMINT CHINESE 10% DEP	3.5
PETITGRAIN PARAGUAY	0.2
ROSE OXIDE RACEMIC 10% DEP	0.5
STYRALLYL ACETATE	0.4
TERPINEOL ALPHA	2.5
TETRAHYDROLINALOL	4.5
total	100.00%

\* Materials of the Invention

**Perfume C: Composition % by weight.**

INGREDIENT	w/w%
ACETYL CEDRENE (Q)	7
AGARBOIS (Q)	15
ALDEHYDE MNA 10% DEP	2.5
BENZYL SALICYLATE (Q)	6.4
cis-JASMONE	1.2
CITRONELLAL	2.2
COUMARIN	1.3
CYCLOPENTADECANOLIDE	6.6
DIHYDROMYRCENOL (Q)	8.5
ETHYLENE BRASSYLATE	2.3
HEXYL CINNAMIC ALDEHYDE	3.5
ISO AMBOIS (Q)	7
ISO BORNYL ACETATE	2.6
LILIAL (G)	5.4
MARENIL (Q)	1.3
MEFROSOL (Q)	6.4
METHYL DIHYDROJASMONATE SUPER (Q)	7.6
PETITGRAIN PARAGUAY	1.2
TERPINEOL ALPHA	3
TETRAHYDROGERANIOL	10
total	100.00%

\* Materials of the Invention

## Perfume D: Composition % by weight.

INGREDIENT	w/w%
4-(5-ETHYLBICYCLO[2.2.1]HEPTYL-2)-CYCLOHEXANOL	1.2
ACETYL CEDRENE (Q)	5.3
ALDEHYDE C11 (UNDECYLENIC ALDEHYDE) 10% DEP	1.4
ALDEHYDE MNA 10% DEP	0.8
ALLYL AMYL GLYCOLATE (Q)	1.3
ARMOISE TUNISIAN	0.2
BANGALOL (Q)	0.3
BENZYL SALICYLATE (Q)	5.1
BERGAMOT OIL	4.8
CEDARWOOD VIRGINIAN OIL	1.1
CITRONELLAL	2
CITRONELLOL	6.9
CYCLOPENTADECANOLIDE	2.3
DIHYDROMYRCENOL (Q)	15.8
ETHYLENE BRASSYLATE	8.8
FENCHYL ACETATE	2.5
HEXYL CINNAMIC ALDEHYDE	5.1
IONONE (Q)	3.5
ISOBORNYL CYCLOHEXANOL	1.8
METHYL DIHYDROJASMONATE SUPER (Q)	5.5
PARA TERT BUTYL CYCLOHEXYL ACETATE	3.4
PARADISAMIDE (Q)	2.8
PEPPERMINT CHINESE 10% DEP	4.3
PHENYLETHYL ALCOHOL	6
ROSE OXIDE RACEMIC 10% DEP	2.1
ROSEACETONE	3.7
TETRAHYDROGERANIOL	2
total:	100.00%

\* Materials of the Invention

## Perfume E: Composition % by weight.

INGREDIENT	w/w%
4-(5-ETHYLBICYCLO[2.2.1]HEPTYL-2)-CYCLOHEXANOL	2.3
AGARBOIS (Q)	4
ALDEHYDE C11 (UNDECYLENIC ALDEHYDE) 10% DEP	1.2
AMBER CORE (Q)	4.3
CARVONE LAEVO (Q) 10% DEP	3.8
CEDARWOOD VIRGINIAN OIL	1.8
cis-JASMONE	0.5
CISTULATE (Q) 10% DEP	0.9
CITRONELLOL	3.6

CORIANDER	0.2
COUMARIN	0.9
DIHYDROMYRCENOL (Q)	4.5
ETHYLENE BRASSYLATE	6.2
FENCHYL ACETATE	3.6
HEXYL CINNAMIC ALDEHYDE	6.8
HEXYL SALICYLATE	7.5
LILIAL (G)	6.5
MARENIL (Q)	2.6
METHYL CHAVICOL	0.4
METHYL DIHYDROJASMONATE SUPER (Q)	3.5
METHYL OCTYL ACETALDEHYDE 10% DEP	5.5
MOSS OAKMOSS SYNTHETIC	0.2
PEPPERMINT CHINESE 10% DEP	3.4
PETITGRAIN PARAGUAY	2.1
PHENYLETHYL ALCOHOL	7.1
TERPINEOL ALPHA	8.4
TETRAHYDROGERANIOL	8.2
TETRAHYDROLINALOL	2
total	100.00%

\* Materials of the invention

#### EXAMPLE 4: PRODUCT BASE EXAMPLES

The following are typical formulations of deodorant products which comprise a perfume or perfume component capable of inhibiting the production of odoriferous steroids. These formulations are made by methods common in the art.

##### **1. Deodorant Sticks**

Ingredient	Content (% by weight)	
	Formulation 1A	Formulation 1B
Ethanol		8.0
Sodium Stearate	7.0	6.0
Propylene glycol	70.0	12.0
Perfume	1.5	2.0
PPG-3 Myristyl ether		28.0
PPG-10 Cetyl ether		10.0
Cyclomethicone		34.0
Water	21.5	

**2. Aerosols**

Ingredient	Content % by weight	
	Formulation 2A	Formulation 2B
Ethanol B	up to 100	
Propylene glycol	as required	
Perfume	2.0	1.2
Chlorhydrrol microdry		31.8
Silicone Fluid DC344		up to 100
Bentone gel IPP		13.65
Dimethyl ether	20.0	
Concentrate		22.0
Water	23.0	

**3. Roll ons**

Ingredient	Content % by weight	
	Formulation 3A	Formulation 3B
Ethanol	to 100%	60.0
Klucel MF		0.65
Cremphar RM410		0.6
Perfume	0.5	1.0
AZTC*	20.0	
Cyclomethicone	68.0	
Dimethicone	5.0	
Silica	2.5	
Water		37.85

\* Aluminium zirconium tetrachlorohydro glycinate

Perfume compositions embodying this invention were made and tested for deodorant action in underarm products, using an Odour Reduction Value test generally as described in US 4 278 658 using the formulations described below.

The Odour Reduction Value test was carried out using a panel of 40 Caucasian male subjects. A standard quantity (approximately 0.25g) of a roll-on product containing one of the perfume compositions or an unperfumed control was applied to the axillae of the panel members in accordance with a statistical design.

After a period of five hours or twenty four hours the underarm odour was judged by three trained female assessors who scored the odour intensity in accordance with a 0 to 5 scale, as shown below:

Score	Odour level	Conc. of aqueous isovaleric acid (m/l)
0	No odour	0
1	Slight	0.013
2	Definite	0.053
3	Moderate	0.22

4	Strong	0.87
5	Very Strong	3.57

Average scores for each test product and the control product were then determined and the score for each test product was subtracted from the score for the control product and the reduction was expressed as a percentage to give the Odour Reduction Value%.

Perfume compositions 'A' to 'E' were all found to exhibit significant deodorant activity.

For example, Perfume 'A' contains 35% of perfume components of the invention. Excluding diluents, this percentage increases to 42.2%. For this perfume the Odour Reduction Value% compared to an unperfumed control was 66.9% (5 hours) and 33.8% at 24 hours.

C237.00/Q

CLAIMS

1. A method for reducing or preventing body malodour by topically applying to human skin a composition comprising an active agent capable of inhibiting the production of odoriferous steroids by micro-organisms on the skin surface, wherein the agent is a perfume component which is capable of inhibiting bacterial 3 $\alpha$ ( $\beta$ )-sterol dehydrogenase and/or steroid 4,5-isomerase.
2. A method according to claim 1 wherein the perfume component is selected from at least one of the following; N-Ethyl-N-(3-methylphenyl)propionamide; 2-Ethyl-N-methyl-N-(3-methylphenyl)butanamide; Dihydromyrcenol; (4-isopropylcyclohexyl)methanol; 3-Methyl-5-phenylpentan-1-ol; 2,2,2-Trichloro-1-phenylethyl acetate; Isobornyl acetate; Allyl amyl glycolate; alpha-Terpineol; Acetyl cedrene; Tetrahydrogeraniol; Citronellal; Cuminic aldehyde; cis-Jasmone; Pine American oil; Peppermint (Chinese); 1,3,3-Trimethyl-2-norbornanol; gamma-Nonalactone; Octahydro-2H-chromen-2-one; cis-4-Decenal; 3-(3-isopropylphenyl)butanal.
3. A method according to claim 1 wherein the composition comprises at least 30% by weight of at least one of the perfume components listed in claim 2.
4. A method for reducing or preventing body malodour by topically applying to human skin a composition comprising a perfume component which inhibits bacterial 3 $\alpha$ ( $\beta$ )-sterol dehydrogenase and/or steroid 4,5-isomerase.
5. A perfume composition comprising a perfume component capable of inhibiting the production of odoriferous steroids by microorganisms on the skin characterised in that the perfume component is capable of inhibiting bacterial 3 $\alpha$ ( $\beta$ )-sterol dehydrogenase and/or steroid 4,5-isomerase.
6. A perfume composition according to claim 5 comprising at least 30% of at least one perfume component capable of inhibiting the production of odoriferous steroids by micro-organisms on the skin characterised in that the perfume component is capable of inhibiting bacterial 3 $\alpha$ ( $\beta$ )-sterol dehydrogenase and/or steroid 4,5-isomerase.
7. The use of a perfume component to reduce body malodour characterised in that the perfume component is capable of inhibiting bacterial 3 $\alpha$ ( $\beta$ )-sterol dehydrogenase and/or steroid 4,5-isomerase.

8. The use of a perfume composition comprising a perfume component to reduce body malodour characterised in that the composition comprises at least 30% by weight of at least one of the perfume components listed in claim 2.
9. A perfume composition comprising at least 30% by weight of one or more of the following perfume components: N-ethyl-N-(3-methylphenyl)propionamide; 2-ethyl-N-methyl-N-(3-methylphenyl)butanamide; dihydromyrcenol; (4-isopropylcyclohexyl)methanol; 3-methyl-5-phenylpentan-1-ol; 2,2,2-trichloro-1-phenylethyl acetate; isobornyl acetate; allyl amyl glycolate; alpha-terpineol; acetyl cedrene; tetrahydrogeraniol; citronellal; cuminic aldehyde; cis-jasmone; pine American oil; peppermint (Chinese); 1,3,3-trimethyl-2-norbornanol; gamma-nonalactone; octahydro-2H-chromen-2-one; cis-4-decenal; 3-(3-isopropylphenyl)butanal.
10. A perfume composition comprising at least 3 of the following perfume components: N-ethyl-N-(3-methylphenyl)propionamide; 2-ethyl-N-methyl-N-(3-methylphenyl)butanamide; dihydromyrcenol; (4-isopropylcyclohexyl)methanol; 3-methyl-5-phenylpentan-1-ol; 2,2,2-trichloro-1-phenylethyl acetate; isobornyl acetate; allyl amyl glycolate; alpha-terpineol; acetyl cedrene; tetrahydrogeraniol; citronellal; cuminic aldehyde; cis-jasmone; pine American oil; peppermint (Chinese); 1,3,3-trimethyl-2-norbornanol; gamma-nonalactone; octahydro-2H-chromen-2-one; cis-4-decenal; 3-(3-isopropylphenyl)butanal.
11. A perfume composition according to claim 10, wherein the perfume composition comprises at least 30% by weight of at least 3 of the specified perfume components.
12. A deodorant product comprising a perfume composition according to any one of claims 5, 9, 10 or 11.

C237.00/Q

Title: Method of Reducing or Preventing MalodourABSTRACT

A method for reducing or preventing body malodour by topically applying to human skin perfume components capable of inhibiting the production of odoriferous steroids by micro-organisms on the skin. The perfume components are capable of inhibiting bacterial  $3\alpha(\beta)$ -sterol dehydrogenase and/or steroid 4,5-isomerase.

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